

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2032195PC/OR	FOR FURTHER ACTION See Form PCT/IPEA/416																									
International application No. PCT/FI2004/000776	International filing date (day/month/year) 17.12.2004	Priority date (day/month/year) 19.12.2003																								
International Patent Classification (IPC) or national classification and IPC See Supplemental Box																										
Applicant Mobidiag Oy et al																										
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of <u>13</u> sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> (sent to the applicant and to the International Bureau) a total of <u>3</u> sheets, as follows:</p> <p><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p> <p>4. This report contains indications relating to the following items:</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%; text-align: center;"><input checked="" type="checkbox"/></td> <td style="width: 15%; text-align: center;">Box No. I</td> <td style="width: 70%;">Basis of the report</td> </tr> <tr> <td style="text-align: center;"><input checked="" type="checkbox"/></td> <td style="text-align: center;">Box No. II</td> <td>Priority</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;">Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td style="text-align: center;"><input checked="" type="checkbox"/></td> <td style="text-align: center;">Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td style="text-align: center;"><input checked="" type="checkbox"/></td> <td style="text-align: center;">Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td style="text-align: center;"><input checked="" type="checkbox"/></td> <td style="text-align: center;">Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;">Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;">Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table>			<input checked="" type="checkbox"/>	Box No. I	Basis of the report	<input checked="" type="checkbox"/>	Box No. II	Priority	<input type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	<input checked="" type="checkbox"/>	Box No. IV	Lack of unity of invention	<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	<input checked="" type="checkbox"/>	Box No. VI	Certain documents cited	<input type="checkbox"/>	Box No. VII	Certain defects in the international application	<input type="checkbox"/>	Box No. VIII	Certain observations on the international application
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Date of submission of the demand 19-10-2005	Date of completion of this report 14-02-2006
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Terese Sandström/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No. PCT/FI2004/000776

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: **Cover sheet**

INTERNATIONAL PATENT CLASSIFICATION (IPC) :

C12Q 1/68 (2006.01)

C12N 15/11 (2006.01)

Box No. I Basis of the report

1. With regard to the language, this report is based on:

the international application in the language in which it was filed

a translation of the international application into _____, which is the language of a translation furnished for the purposes of:

international search (Rules 12.3(a) and 23.1(b))

publication of the international application (Rule 12.4(a))

international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the elements of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

the international application as originally filed/furnished

the description:
pages 1 - 24 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

the claims:
pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 25 - 27 received by this Authority on 03 - 02 - 2006
pages* _____ received by this Authority on _____

the drawings:
pages 1 - 2 (figures 1 - 3) as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. The amendments have resulted in the cancellation of:

the description, pages _____
 the claims, Nos. _____
 the drawings, sheets/figs _____
 the sequence listing (*specify*): _____
 any table(s) related to the sequence listing (*specify*): _____

4. This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

the description, pages _____
 the claims, Nos. _____
 the drawings, sheets/figs _____
 the sequence listing (*specify*): _____
 any table(s) related to the sequence listing (*specify*): _____

* If item 4 applies, some or all of those sheets may be marked "superseded."

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:
 - a. type of material
 - a sequence listing
 - table(s) related to the sequence listing
 - b. format of material
 - on paper
 - in electronic form
 - c. time of filing/furnishing
 - contained in the international application as filed
 - filed together with the international application in electronic form
 - furnished subsequently to this Authority for the purposes of search and/or examination
 - received by this Authority as an amendment* on _____
2. In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

* If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

10/583329
International application No.

PCT/FI2004/000776

Box No. II Priority

AP3 Rec'd PCT/PTO 16 JUN 2007

1. This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
 - copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
 - translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

The priority document has been received and examined.

Box No. IV Lack of unity of invention

PCT Rec'd PCT/PTO 16 JUN 2005

1. In response to the invitation to restrict or pay additional fees the applicant has, within the applicable time limit:
 - restricted the claims
 - paid additional fees
 - paid additional fees under protest and, where applicable, the protest fee
 - paid additional fees under protest but the applicable protest fee was not paid
 - neither restricted the claims nor paid additional fees
2. This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:
 - complied with
 - not complied with for the following reasons:

The following separate inventions were identified:

1: Claims 1-11 and 16 directed to detection and identification of bacterial species involving the use of the universal primer pair SEQ. ID. NOs. 20 and 21 in combination with species-specific probes. Both the primers and probes hybridize to the rpoB gene.

2-12: Claims 12-15 directed to the species-specific probes SEQ. ID. NO. 1-19, which hybridize to a hyper-variable region that is situated near conserved regions in the rpoB gene. (One invention for each bacterial species that the probes may identify, i.e. one invention for the two probes capable of identifying *H.influenzae*, one invention for the two probes capable of identifying *S.pyogenes* etc.)

The present application has been considered to contain 12 inventions which are not linked such that they form a single general inventive concept, as required by Rule 13 PCT for the following reasons:

.../...

4. Consequently, this report has been established in respect of the following parts of the international application:

- all parts
- the parts relating to claims Nos. 1-11 and 16

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box IV

The closest prior art has been identified as:

D1: WO0192573 A1
D2: US5786147 A
D3: WO0131061 A1
D4: WO9533851 A2
D5: WO03008645 A1
D6: WO03020972 A1
D7: WO03016534 A1

D1 discloses a method for identifying a *Mycobacterium* species using a combination of a universal primer pair and a species-specific probe, all hybridizing to the *rpoB* gene. (Page 5, line 21-25; page 5, line 29-page 6, line 7; page 7, line 16-page 8, line 4; table 2.)

Similar methods for combining universal primers with species-specific probes are disclosed in D2-D7. (D2: column 4, line 56-column 11, line 20; examples 1-2; D3: page 5, lines 1-18; page 7, line 15-page 10, line 8; page 13, line 6-page 21, line 23; D4: page 5, lines 3-13; page 15, lines 8-31; page 19, lines 6-17; table 2; claims; D5: page 5, line 22-page 9, line 15; page 11, line 15-page 20, line 8; D6: abstract; page 6, line 19-page 8, line 15; claims; D7: page 6, line 13-page 13, line 23; example 1.)

Invention 1:

From a comparison of the disclosure of D1-D7 and the technical features of claims the following technical features can be seen to make a contribution over this prior art: The use of the specific primer pair SEQ. ID. NO. 20 and 21 in combination with species-specific probes for identifying and detecting bacterial species.

This feature is hence considered as special technical feature in the sense of Rule 13.2 PCT.

The effect of this feature is an alternative method for detecting and identifying bacterial species.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box IV

Inventions 2-12:

From a comparison of the disclosure of D1-D7 and the technical features of claims the following technical features can be seen to make a contribution over this prior art: Species-specific probes, disclosed in SEQ. ID. NOS. 1-19, for a number of different bacterial species.

This feature is hence considered as special technical feature in the sense of Rule 13.2 PCT.

The effect of this feature is species-specific probes for a number of different species. (One invention for each bacterial species that the probes may identify, i.e. one invention for the two probes capable of identifying *H.influenzae*, one invention for the two probes capable of identifying *S.pyogenes* etc.)

Even though the probes in inventions 2-12 might be used in the method in invention 1, they can not be considered "specially designed" for carrying out the method in invention 1, as other probes in the region are previously known. In addition, these probes can be used for any hybridization assay and the product claims do not imply a limitation as to a particular use.

The above analysis shows that the special technical features of invention 1 are neither the same as, nor corresponding to, those of inventions 2-12.

Consequently, neither the objective problem underlying the subjects of the claimed inventions, nor their solutions defined by the special technical features allow for a relationship to be established between the said inventions, which involves a single general inventive concept.

In conclusion, therefore, the claims are not linked by same or corresponding special technical features and define different inventions not linked by a single general inventive concept.

The application, hence does not meet the requirements of unity of invention as defined in Rule 13.1 and 13.2 PCT.

Since no fee was paid for a complete search, only invention 1 was searched during the Chapter I procedure. Hence only invention 1 is examined in this report.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/FI2004/000776

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	<u>1-11, 16</u>	YES
	Claims	_____	NO
Inventive step (IS)	Claims	<u>1-11, 16</u>	YES
	Claims	_____	NO
Industrial applicability (IA)	Claims	<u>1-11, 16</u>	YES
	Claims	_____	NO

2. Citations and explanations (Rule 70.7)

Documents cited in the International Search Report:

D1: WO0192573 A1

D2: US5786147 A

D3: WO0131061 A1

D4: WO9533851 A2

D5: WO03008645 A1

D6: WO03020972 A1

D7: WO03016534 A1

D8: Khamis A. et al., "Usefulness of *rpoB* Gene Sequencing for Identification of *Afipis* and *Bosea* Species, Including a Strategy for Choosing Discriminative Partial Sequences", *Applied and Environmental Microbiology*, November 2003, pages 6740-6749

D9: Dahllöf I. et al., "rpoB-Based Microbial Community Analysis avoids Limitations Inherent in 16S rRNA Gene Intraspecies Heterogeneity", *Applied and Environmental Microbiology*, August 2000, pages 3376-3380

D10: Mollet C. et al., "rpoB sequence analysis as a novel basis for bacterial identification", *Molecular Microbiology*, 1997, Vol. 26, No. 5, pages 1005-1011

The present application relates to diagnosis of bacterial infections based on species-specific probes and broad-range primers. The species-specific probes originate from hyper-variable regions situated near the conserved sequences of the gene region encoding for RNA polymerase beta subunit, *rpoB*, of infection causing bacteria. The broad-range primers originate from conserved regions in the same gene. The problem to be solved by the present invention is to find a method for sensitive, effective, and species-specific identification of only the desired bacterial species among various bacteria, which may be present in a clinical sample.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V

D1 discloses a method for identifying a *Mycobacterium* species using a combination of a universal primer pair and a species-specific probe. All hybridize to the *rpoB* gene. The universal primer pair gives rise to a PCR fragment of 157 base pairs. (Page 5, lines 21-25; page 5, line 29-page 6, line 7; page 7, line 16-page 8, line 4; table 2.)

D2 relates to detection and amplifying techniques utilizing oligonucleotide probes and primer and to the applications of these oligonucleotides for detecting enterobacteria. The methods are based on the use of nucleic acid sequences, which are strongly conserved for the enterobacterial species alone. These sequences are specifically defined in the *rpoB* gene, thereby enabling the enterobacterial family to be distinguished from other bacterial families. (Column 4, line 56-column 11, line 20; examples 1-2.)

Similar method for combining universal primers with species-specific probes are disclosed in D3-D7 (D3: page 5, lines 1-18; page 7, line 15-page 10, line 8; page 13, line 6-page 21, line 23; D4: page 5, lines 3-13; page 15, lines 8-31; page 19, lines 6-17; table 2; claims; D5: page 5, line 22-page 9, line 15; page 11, line 15-page 20, line 8; D6: abstract; page 6, line 19-page 8, line 15; claims; D7: page 6, line 13-page 13, line 23; example 1.) All these documents disclose methods for detection, identification and/or resistance analysis within single family only.

None of the cited documents disclose a method suitable for identification of a large number of bacterial pathogens simultaneously, i.e. the screening of a clinical specimen for a disease-causing bacteria. The primers disclosed in the cited prior art are derived from the species-specific regions of the *rpoB* gene or are derived from the antibiotic resistance genes and do not enable the detection of several bacterial species, which may be distantly related. Furthermore, none of the documents disclose a method, which is useful in bacterial diagnosis of infectious diseases, especially those causing respiratory tract infections and ear, nose and throat diseases.

The subject matter claimed in claims 1-11 and 16 differs from D1-D7 due to the specific broad-range primers disclosed in sequences SEQ. ID. NOS. 20 and 21. Hence, the subject matter claimed in claims 1-11 and 16 is novel.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V

These broad-range primer sequences have been based on a comparison of *rpoB* sequences from a large number of different bacteria, thereby having a broader detection range than the primers disclosed in D1-D7. With the broad-range primers of the present invention, the amplification of *rpoB* genes from phylogenetically different bacterial species is successful regardless of whether the bacterial species are gram-negative or gram-positive, even in the presence of large amount of human normal flora DNA. With these specific primers, it is possible to diagnose an infectious disease with unknown causative agent. In addition these primers give rise to a smaller PCR product, thereby increasing the effectiveness of the analysis.

Even though specific primers originating from conserved sequences of *rpoB* have been disclosed in the prior art, the development of a specific and sensitive diagnostic method for detecting and identifying bacterial species causing infection from a clinical sample cannot be considered as routine procedure for a skilled person, since unpredictability is highly characteristic in the field of biology and gene technology. Many factors affect the detection of a specific microorganism in a clinical sample, which contains DNA from many different sources. For instance, all biological samples of human origin contain DNA from normal flora. The effect of the normal flora on the analysis method cannot be predicted. Its effect can be unpredictable in cases, where the detection and identification of microorganisms, such as those causing infections, is base on conserved genes present in both eukaryotic and prokaryotic organisms. Additionally, clinical samples of human origin always contain human DNA, which similarly is a "contaminant" in cases where the detection and identification of microorganisms, such as those causing infections, is based on universally conserved genes.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V

In view of the above, the subject matter claimed in claims 1-11 and 16 is considered to involve an inventive step.

D8-D10 are considered to disclose the general state of the art.

To summaries, the subject matter claimed in claims 1-11 and 16 is novel and is considered to involve an inventive step. The subject matter claimed in claims 1-11 and 16 is considered to be industrially applicable.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/FI2004/000776

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO2004041841 A2	21.05.2004	04.11.2003	05.11.2002

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure (day/month/year)	Date of written disclosure referring to non-written disclosure (day/month/year)